the thickness of the section. Nevertheless, image cytometry is similar to conventional microscopic analysis, and so it is possible for the operator to identify each measured nucleus. Thus, while the DNA histogram may lack the resolution of flow, it can be relied on to represent only malignant cells and to be free of contamination by nontumorous cells.

DNA histograms are analyzed first for DNA ploidy by locating the major peak of the histogram—the DNA content shared by most of the cells. If this value is the same as that for normal tissues, then the tumor is DNA diploid; otherwise, it is DNA aneuploid, with a DNA index that is the ratio of the tumor value to the normal value. Flow or image cytometry reliably detects a deviation of 10% from normal.

Further analysis of the DNA histogram estimates the fraction of tumor cells that are in the S phase (synthesizing DNA). The DNA content of S-phase cells is intermediate between the major histogram peak (postdivision resting cells) and the peak located at twice this DNA content (predivision and dividing cells). The S-phase fraction relates directly to the rate of cellular proliferation—the higher the fraction, the more rapidly the tumor is growing. Estimating the S-phase fraction from the DNA histogram is difficult, however, and the results become unreliable if the preparation contains much nuclear debris or if the tumor has aneuploid populations of cells.

The usefulness of DNA cytometry in evaluating most solid tumors is extensively documented in studies using either flow or image cytometry. Many of these studies are retrospective, using archival paraffin-embedded tissue blocks. Thick (50 μm) sections are cut from the block and then are dissociated to obtain isolated nuclei for either flow or image cytometry. Such studies show that DNA aneuploidy and high S-phase fractions are usually associated with tumors of high clinical stage, high histopathologic grade, and generally poor prognosis. The independent diagnostic and prognostic powers of DNA cytometry become particularly important in low-stage tumors. For example, even low-stage breast, thyroid, or prostate cancers that show DNA aneuploidy or a high S-phase fraction have a high probability of establishing distant metastases whereas comparable DNA-diploid, low S-phase tumors are unlikely to metastasize. The results of DNA cytometry then provide clinicians with relevant information about the potential aggressiveness of the cancer.

In summary, DNA cytometry provides important diagnostic and prognostic information for the evaluation of solid tumors. This is most clearly established for low-stage breast cancers but also is well documented for a broad range of solid tumors. DNA cytometry is not infallible, however. As with all new tests, its power and limitations should be clearly understood, and results should be interpreted cautiously within the context of the overall clinical and histopathologic picture.

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Relationship of Amyloid to Alzheimer's Disease

THE CLINICAL DIAGNOSIS of Alzheimer's disease or senile dementia of Alzheimer's type is confirmed in suspected cases by a neuropathologist after a careful examination of brain sections after necropsy. Of the microscopic lesions used to confirm the clinical diagnosis, all are present in normal aged brains but occur in exaggerated number and severity in the brains of patients with Alzheimer's disease. Three of these microscopic criteria have the classic tinctorial properties associated with β-pleated sheet fibrils, or amyloid. These are senile plaques, neurofibrillary tangles, and cerebral amyloid angiopathy or cerebrovascular amyloidosis. Of these, senile plaques and amyloid angiopathy are biochemically related, whereas the amyloid of neurofibrillary tangles appears to be composed of a different peptide. Whether the amyloidotic peptides found in excess in the brains of patients with Alzheimer's disease are a cause of the disease or simply an epiphenomenon is not clear. Nevertheless, brain amyloid is considered a marker of Alzheimer's disease or senile dementia of Alzheimer's and its presence important in confirming the diagnosis pathologically.

Senile plaque and microvascular amyloid are biochemically similar if not identical and consist of a unique peptide with a molecular weight of about 4,200. Since 1987, molecular biologic techniques have been used extensively to show that this peptide (known as the A4 or β-peptide) derives from a large precursor molecule composed of almost 700 amino acids. The identity of senile plaque and microvascular amyloid has been confirmed both biochemically and indirectly using immunohistochemical techniques at the light and ultrastructural levels. Some have suggested that immunohistochemistry using antibodies to the Alzheimer A4 or β-peptide is more sensitive in showing the earliest lesions of Alzheimer's disease than conventional silver stains or Congo red or thioflavin methods. Immunohistochemical studies have also shown that A4 or β-peptide is widely distributed in the brain, being found in areas not traditionally thought to be affected by the neuropathologic changes of Alzheimer's disease, such as the deep central grey matter, brain stem, and cerebellum, and it may even be seen in the gut and skin.

Current research on the biochemistry and molecular biology of Alzheimer A4 or β-peptide amyloid focuses on several areas. An intensive effort is aimed at establishing how the peptide is cleaved from its precursor (the A4P or APP molecule) and whether it originates in the brain, the peripheral circulation, or both. Although A4 or β-peptide is the most commonly observed amyloid in the central nervous system of patients with Alzheimer's disease or senile dementia of the Alzheimer's type, small amounts of γ-trace peptide (usually associated with amyloid angiopathy in Icelandic patients who have familial cerebral hemorrhage) have recently been found in the walls of the amyloidotic arterioles of patients with Alzheimer's disease. The biologic importance of this finding remains to be determined. Ideally, patients with Alzheimer's

disease might be segregated from patients with non-Alzheimer's dementia by the finding of elevated levels of A4 or β-peptide in the blood or cerebrospinal fluid—that is, a simple biochemical test could be used to confirm the presence of Alzheimer's disease. This is now done inferentially from clinical data and imaging studies, and the autopsy is used for definitive confirmation.

The A4 protein has been shown to be accumulated within lysosomes of certain neuronal populations in the brain, suggesting that proteolysis of the precursor with resultant deposition of the A4 or β-peptide within tissues may be a pivotal event in the neuronal degeneration that characterizes Alzheimer's disease both clinically and pathologically. In any event, research into possible mechanisms of processing of the A4 peptide into an insoluble form in the brain may lead to therapeutic strategies by which this processing can be engineered so that smaller amounts of microvascular and senile plaque amyloid are ultimately deposited within brain parenchyma. It remains an open question whether this will lead to stabilization or amelioration of the clinical signs and symptoms of Alzheimer's disease or Alzheimer's senile dementia.

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Stereotactic Brain Biopsy

THE MANAGEMENT OF MASS LESIONS of the brain continues to evolve with the increasing availability of stereotactic biopsy. Stereotactic biopsy is done through a small drill hole in the skull under local anesthesia and carries an extremely small risk of complications. The total time for obtaining a biopsy specimen is about two hours. Most patients come in for the procedure in the morning and leave the hospital after an overnight observation period. Stereotactic biopsy is capable of procuring tissue from virtually any location within the cranial cavity, including the third ventricle, hypothalamic region, brain stem, and posterior fossa. The availability of stereotactic biopsy allows the histologic diagnosis of any brain mass.

Basic mathematic principles are used to identify any point within the cranial cavity by means of three coordinates. The stereotactic instruments provide the three coordinates that guide the biopsy forceps to a target point determined on the radiologic image. With experience, representative material is procured in virtually all cases.

The pathologic interpretation is limited by the small size of the specimen, however. The accuracy of histologic diagnosis in experienced hands is about 90% overall. The use of smears is highly recommended as the primary means of making a diagnosis.

The accuracy and specificity of diagnosis are greatest for regionally homogeneous neoplastic lesions such as metastatic carcinoma, craniopharyngioma, oligodendroglioma, and malignant lymphoma. In these cases, the diagnosis depends only on the neurosurgeon obtaining tissue from the lesion and the pathologist making the correct diagnosis.

In regionally heterogeneous neoplasms such as astrocytomas and pineal germ cell neoplasms, the small size of the specimen creates theoretical problems. Here the diagnosis depends not only on obtaining abnormal material and the pathologic interpretation but also on regional differences within the neoplasm. Two solutions are available at stereotactic biopsy to overcome this problem. The first is to take several specimens from different target points in the lesion, increasing the tissue volume to provide a representative sample. This adds to the time and risk of the procedure but is an acceptable solution. The second is to interpret the biopsy in conjunction with full clinical and radiologic data to arrive at a clinicopathologic diagnosis. We use the latter method, and it has been our experience that a single specimen from an astrocytic neoplasm provides an accurate placement of the lesion in a three-tiered classification that is adequate for appropriate clinical management. The use of a single biopsy specimen in conjunction with tumor markers and radiologic features is adequate for most pineal germ cell neoplasms.

The lowest accuracy rate for stereotactic biopsy is attained when the biopsy specimen shows inflammation. Most of these will be nonneoplastic lesions. The rate of identifying a specific cause is low and requires careful handling of the tissue for immunohistochemistry (viral antigens), electron microscopy (viral particles), culture, and polymerase chain reaction (viral nucleic acid). Although a stereotactic biopsy specimen that shows inflammation is rarely from a neoplasm, we have had specimens interpreted as nonspecific inflammation turn out on a subsequent specimen to be malignant lymphoma, Hodgkin's disease, pineal germinoma, and anaplastic astrocytoma. It is prudent, therefore, to carefully evaluate inflammatory lesions diagnosed on stereotactic biopsy for the possibility of a neoplasm.

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Micrometastases in Melanoma

THE FIVE-YEAR SURVIVAL RATE for patients with melanoma spread to the regional nodes, the first site of metastasis for most melanoma patients, varies from 20% to 50% according to the number of nodes that contain metastatic melanoma and the micrometer measured thickness of the primary tumor.